Remote and local ischemic postconditioning further impaired skeletal muscle mitochondrial function after ischemia-reperfusion

Ziad Mansour, MD, PhD,[a,b] Anne L. Charles, PhD,[b,c] Jamal Bouitbir, PhD,[b,c] Julien Pottecher, MD,[b,d] Michel Kindo, MD, PhD,[a,b] Jean-Philippe Mazzucotelli, MD, PhD,[a] Joffrey Zoll, PhD,[b,c] and **Bernard Geny, MD, PhD,[b,c]** *Strasbourg, France*

Objective: **Muscular injuries contribute to perioperative and long-term morbidity after vascular surgery in humans. We determined whether local and remote ischemic postconditioning might similarly decrease muscle mitochondrial dysfunction through reduced oxidative stress.**

Methods: **Eighteen male Black-6 mice were divided in three groups: (1) sham mice had no ischemia (sham), (2) ischemia-reperfusion (IR) mice underwent 2-hour tourniquet-induced ischemia on both hind limbs, followed by 2-hour reperfusion, and (3) postconditioning (PoC) mice underwent four bouts of 30-second reperfusion and 30-second ischemia at the onset of reperfusion on the right limb; thus, the right limb underwent local PoC and left limb underwent remote PoC (rPoC). Maximal oxidative capacity (V_{max}) of the gastrocnemius muscle mitochondrial respiratory chain was measured. Oxidative stress was evaluated by dihydroethidium staining. Expressions of genes involved in antioxidant defense (*superoxide dismutase* [*SOD1*], *SOD2*, *glutathione peroxidase* [*GPx*]), apoptosis (*Bax*, *BclII*), and inflammation (*interleukin-6*) were determined by quantitative real-time polymerase chain reaction. Muscle inflammation was determined using immunohistochemistry.**

Results: **IR reduced V_{max} (8.5 ± 2.2 vs 10.2 ± 1.8 μmol O_2/min/g dry weight; $P = .034$), and increased dihydroethidium staining (134.8%; $P = .039$). IR decreased GPx expression (−47.9%; $P = .048$) and increased the proapoptotic marker Bax (255.5%; $P = .020$). Local PoC and rPoC further increased these deleterious effects. PoC decreased V_{max} to 4.4 ± 1.4 μmol O_2/min/g dry weight (sham vs PoC, −56.9% [$P < .001$]; IR vs PoC, −48.2% [$P < .001$]). rPoC similarly reduced V_{max} to 5.1 ± 1.9 μmol O_2/min/g dry weight (sham vs PoC, −50.0% [$P < .001$]; IR vs PoC, −40.0% [$P = .001$]). Dihydroethidium staining was further increased by PoC (207.2%; $P = .002$) and rPoC (305.4%; $P < .001$) compared with sham and was associated with macrophage infiltration. Local PoC increased *SOD1*, *SOD2*, and the antiapoptotic *Bcl-2*, and rPoC increased *Bax* (391.6%; $P < .001$) and the *Bax/BclII* ratio (621.7%; $P < .001$).**

Conclusions: **Local and remote ischemic postconditioning further increased injury by enhancing mitochondrial dysfunction, oxidative stress production, and inflammation. Caution should be applied when considering ischemic postconditioning in vascular surgery. (J Vasc Surg 2012;56:774-82.)**

Clinical Relevance: **For the first time in the setting of hind limb ischemia-reperfusion, ischemic postconditioning not only failed to protect skeletal muscle but also increased injury by enhancing mitochondrial dysfunction, oxidative stress, and inflammation. Although unexpected, these data are in line with recent reports in patients showing that ischemic postconditioning did not reduce infarct size or improve myocardial function recovery and might have a potential harmful effect. Thus, our data are likely to be pertinent in patients who need major vascular surgery and strongly suggest that caution should be taken before a broader translation of ischemic postconditioning into surgical daily practice.**

Restoration of blood flow to ischemic tissue is vital and a prerequisite to interrupt the development of irreversible damage. This holds true for all organs and is particularly interesting in the setting of vascular surgery. Indeed, even subtle intraoperative skeletal muscle ischemia contributes to remote organ injuries, thus contributing to perioperative and long-term morbidities.[1-4] However, reperfusion per se is also deleterious, and reperfusion-induced injury is thought to participate in up to 40% of muscle damage.[5]

Several approaches have been proposed to reduce reperfusion-related injuries. Ischemic preconditioning consistently reduced skeletal muscle damage, decreased mitochondrial dysfunction, enhanced limb protection, and reduced remote organs alterations in experimental animals and in humans.[2,6-8] Nevertheless, except during surgery, the occurrence of ischemia is difficult to predict, which might limit a broader use of ischemic preconditioning.

From the Service de Chirurgie Cardiovasculaire, Pôle de Cardiologie, Hôpitaux Universitaires, CHRU de Strasbourg[a]; Equipe d'Accueil 3072, Institut de Physiologie, Faculté de Médecine, Université de Strasbourg[b]; Service de Physiologie et d'Explorations Fonctionnelles, Pôle de Pathologie Thoracique, CHRU de Strasbourg[c]; and Pôle Anesthésie Réanimation Chirurgicale, SAMU, Hôpitaux Universitaires de Strasbourg.[d]

Financial aid was received from the Cardiovascular Operated Patients Association (ONCOVAS).

Author conflict of interest: none.

Additional material for this article may be found online at www.jvascsurg.org.

Reprint requests: Prof Bernard Geny, Service de Physiologie et d'Explorations Fonctionnelles et Equipe d'Accueil 3072, Université et Hôpitaux Universitaires de Strasbourg, 67091 Strasbourg, France (e-mail: bernard.geny@chru-strasbourg.fr).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214/$36.00

doi:10.1016/j.jvs.2012.01.079

Controlled reperfusion thus appeared as a valuable therapeutic tool after limb ischemia.[9] Local ischemic postconditioning (PoC), defined as the application of repeated cycles of reperfusion and ischemia performed on the ischemic limb at the onset of reperfusion, is thought to be safe and easy to perform.[7,10] Although data are still scarce in the setting of vascular surgery-induced skeletal muscle ischemia, a beneficial effect of local PoC has been demonstrated, including effective functional protection.[10-14] Nevertheless, local PoC might directly stress the target organ and further impair the vessels involved in the current ischemia-reperfusion (IR) episode, suggesting that remote PoC (rPoC) might be advisable.[15] Indeed, remote ischemic preconditioning, defined as an ischemic conditioning stimulus applied before an injurious ischemic episode but at a remote site, appeared not harmful for the ischemic territory and afforded an equivalent protection compared with local ischemic preconditioning in heart and skeletal muscles.[16,17]

To gain insights into the mechanisms involved in the potential beneficial effects of both local PoC and rPoC, and in view of the central role of mitochondria and oxidative stress in cell function and survival after major trauma and IR,[18] we aimed to determine skeletal muscle mitochondrial function and production of reactive oxygen species (ROS) and inflammation in the setting of limb IR, associated or not with PoC and rPoC. Mitochondria are the main energy source of the cells, converting nutrients into energy through cellular respiration, and mitochondrial dysfunction is associated with cell dysfunction and, ultimately, with organ impairment. Consistently, IR induced skeletal muscle mitochondrial dysfunctions in animals and humans,[6,14,19] and more important, improving skeletal muscle mitochondrial function enhanced walking capacities in healthy individuals and in patients suffering from peripheral arterial disease.[20,21]

Increased oxidative stress also largely participated in IR deleterious effects. We and others recently demonstrated that the beneficial effects of local ischemic PoC or superoxide dismutase (SOD) mimetic was associated with a reduced ROS production and an enhanced antioxidant defense in experimental models of lower limb IR.[14,22]

The objective of this study was therefore to challenge the hypothesis that local PoC and rPoC reduce IR-induced mitochondrial respiratory chain injury in skeletal muscles through decreased ROS production and inflammation. We also investigated whether PoC might further increase muscle impairment through prolonged and repeated ischemia.

METHODS

Procedures were conducted in accordance with *Guide for the Care and Use of Laboratory Animals* (U.S. National Institutes of Health publication No. 85-23, National Academy Press, Washington DC, revised 1996).

Animals. Experiments were performed on adult (8-10 weeks old) male Black-6 mice (Depré, France) weighing 20 to 24 grams. Animals were housed in a neutral temperature environment (22 ± 2°C) on a 12/12-hour light/dark cycle and were provided food and water ad libitum.

Preoperative management. Mice were placed in a hermetic anesthetic induction cage and ventilated with gas mixture of 4% isoflurane (AERRANE; Baxter S.A.S., Maurepas, France) and oxygen. After induction, mice were placed on heating blankets (MINERVE, Esternay, France), with a preselected 35°C temperature maintained during the procedure. Spontaneous ventilation was allowed through an oxygen-delivering mask, with different concentrations of isoflurane depending on the surgical stage (2% during painful stimuli, and 1% during latent periods).

Experimental design. A tourniquet was placed around each groin without any skin incision. Mice were divided into three groups of six mice each. The control group (sham) was sham operated and had no ischemia. In the IR group, tourniquets were tightened to induce a 2-hour ischemic assault in both hind limbs. Then, a 2-hour reperfusion period was allowed before gastrocnemius muscle in both hind limbs was harvested. As previously reported,[23] visual assessment confirmed the lack of vascularization during ischemia and the occurrence of reperfusion when the rubber band was removed. Although a tourniquet may only mimic surgery, stopping both arterial and venous blood flows and allowing lack of venous flow to potentially increase the ischemic lesions, such an approach appears clinically pertinent because tourniquet ischemia is widely used in surgery of the lower extremities to reduce blood loss and to improve the surgical field.[24] Furthermore, although visual assessment can be taken in default, recent data investigating local lactate levels support that this mouse model allows significant IR episodes.[25]

In the PoC group, four PoC cycles were performed on the right hind limb at the onset of reperfusion. Each cycle consisted of 30 seconds of reperfusion, followed by 30 seconds of ischemia. Thus, the right hind limb received local PoC and the left hind limb received rPoC. After PoC, both hind limbs had 2 hours of reperfusion, as in the IR group (Fig 1).

At the end of the experiment, the right and left gastrocnemius muscle were dissected, adipose and connective tissues were excised, and mitochondrial respiration studies were immediately performed. Part of the muscles were frozen in liquid nitrogen-cooled isopentane and stored at −80°C for histologic analysis and messenger RNA (mRNA) expressions.

Activity of muscle mitochondrial respiratory chain complexes. Measuring oxygen consumption in skinned fibers is a unique technique to determine the maximum functional oxidative capacity (V_{max}) of the skeletal muscle in its cellular environment. The maximal mitochondrial respiratory rate was studied in saponin-skinned fibers of white gastrocnemius through oxygen consumption measurement by using a Clark-type electrode (Strathkelvin Instruments, Glasgow, UK), as previously described.[14,26,27]

Dihydroethidium staining. To investigate the redox state of the skeletal muscles, depending on ROS production, serial sections (10-μm thick) were cut on a

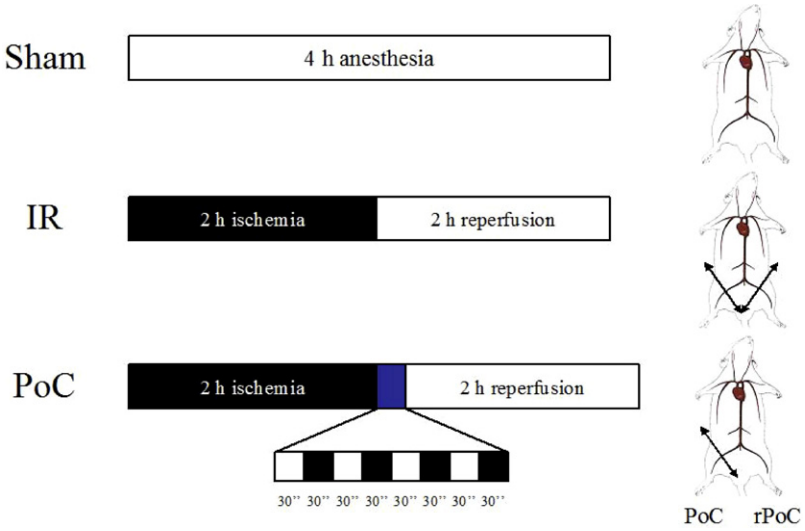

Fig 1. Experimental design. Sham animals underwent 4 hours of general anesthesia and a sham operation. The ischemia-reperfusion (*IR*) animals underwent 2 hours of tourniquet-initiated ischemia in both hind limbs (*dark bar*), followed by 2 hours of reperfusion (*open bar*). The ischemic postconditioned (*PoC*) animals underwent the same IR protocol, but reperfusion on the right hind limb was preceded by four 30-second consecutive reperfusion sequences, each separated by 30 seconds of reocclusion. The right hind limb thus underwent local PoC and the left hind limb underwent remote PoC (*rPOC*).

cryostat microtome and thaw-mounted into glass slides. After air-drying, slides were incubated for 30 minutes at 37°C with 2.5 μmol/L dihydroethidium (DHE) in phosphate-buffered saline (PBS). DHE produces red fluorescence when oxidized to ethidium bromide.[14] After staining, sections were rinsed, air dried, mounted in Vectashield (Vector Laboratories Inc, Burlingame, Calif), and cover slipped. They were examined under an epifluorescence microscope (Eclipse E800; Nikon, Melville, NY; original magnification, ×20), and the emission signal was recorded with a Zeiss 573- to 637-nm filter (Carl Zeiss, München-Hallbergmoos, Germany).

Quantitative real-time polymerase chain reaction. As previously reported,[17] total RNA was obtained from muscles using Trizol reagent (Invitrogen Life Technologies, Rockville, Md). RNA was stored at −70°C until the reverse transcription reaction was performed. Complimentary (c)DNA was synthesized from total RNA (2 μg) with the iScript cDNA Synthesis kit (Bio-Rad, Hercules, Calif). To perform the real-time (RT) polymerase chain reaction (PCR), 1 μL of cDNA was used in a final volume of 20 μL, containing 10 μmol/L of each primer (sense and antisense), SYBR green (Invitrogen Life Technologies, Rockville, Md) as the fluorescent dye, and water. The RT-PCR measurement of individual cDNA was performed in triplicate using SYBR green dye to measure duplex DNA formation with the LightCycler System (Roche Diagnostics, Meylan, France). Primer sequences were designed using information contained in the public database in the GenBank of the National Center for Biotechnology Information.

Quantification of gene expression was done using the *β-actin* gene as the inner control, which is the most stable gene for reverse transcription RT-PCR measurements. Relative mRNA levels of genes involved in antioxidant defense (*SOD1*, *SOD2*, *glutathione peroxidase* [*GPx*]), apoptosis (*Bax* and *BclII*), and inflammation (*interleukin-6*) were calculated using the ΔΔCT method. Sequences of the primer sets used are listed in Table I (online only).

Immunohistochemical procedure. To detect the inflammation in the gastrocnemius, we used monocyte macrophage-2 (Chemicon, AbCys, Paris, France) antibody on serial sections (10-μm thick) of muscles. Briefly, muscle sections were air-dried, and fixed in paraformaldehyde 4% for 3 minutes. They were placed in a 2% solution of hydrogen peroxide for 5 minutes, which served to reduce endogenous or pseudoperoxidase background staining. Primary antibody was used at predetermined optimal dilutions. A standard indirect immunoperoxidase procedure using biotinylated goat antirat antibody (Chemicon, AbCys) and streptavidin–horseradish peroxidase (Chemicon, AbCys) was used to detect binding of monocyte macrophage-2. The slides were developed by diaminobenzidine substrate. Tissues were counterstained briefly with hematoxylin and eosin before mounting.

Statistical analysis. SPSS 17.0 software (SPSS Inc, Chicago, Ill) was used for statistical analyses, and graphics were generated by GraphPad Prism 4 software (GraphPad Software Inc, San Diego, Calif). Means were compared between groups using one-way analysis of variance with the least significant differences post-test. Results are shown as

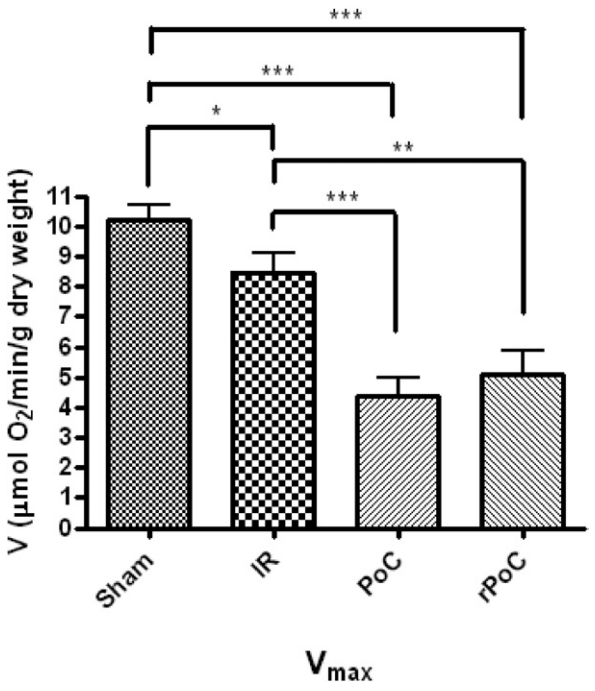

Fig 2. Maximal mitochondrial respiratory rate in sham, after ischemia-reperfusion (*IR*), and after local postconditioning (*PoC*) and remote ischemic PoC (*rPoC*). Maximal oxidative capacity (V_{max}) was assessed using glutamate-malate as the mitochondrial substrate. Results are expressed as means ± standard error of the mean (*error bar*). *$P < .05$. **$P < .01$. ***$P < .001$.

mean ± standard error (± standard error of the mean in graphics). Results with a $P < .05$ were considered statistically significant.

RESULTS

Effects of IR on skeletal muscle mitochondrial respiratory function, ROS production, antioxidant defense, and inflammation. IR impaired the mitochondrial respiratory chain, decreasing significantly skeletal muscle V_{max} (10.2 ±1.8 vs 8.5 ±2.2 µmol O_2/min/g dry weight; $P = .034$, in sham and IR groups, respectively; Fig 2).

To examine the redox state of the cell and implication of oxidative stress, DHE staining was used as an approach of ROS production. In gastrocnemius muscles, IR increased significantly the level of fluorescence, from 4370 ± 924 in sham to 10,258 ± 5351 in IR (134.8%; $P = .039$), supporting an increased presence of superoxide anion (Fig 3). Concerning the antioxidant defense, IR tended to decrease *SOD1* (−39.4%; $P = .375$) and *SOD2* (−31.6%; $P = .394$) and significantly decreased *GPx* expression (−47.9%; $P = .048$; Table II, online only; Fig 4).

We also determined the effects of IR on the expression of genes involved in apoptosis. IR increased the expression of the proapoptotic *Bax* gene (255.5%; $P = .020$; Table II, online only; Fig 5). Our investigation of inflammation showed that IR did not increase *interleukin-6* expression or monocyte-macrophage infiltration (Table II, online only; Fig 6).

Effects of local PoC and rPoC on IR-induced deleterious effects. Local ischemic PoC further impaired the mitochondrial respiratory chain. PoC decreased V_{max} −56.9% (to 4.4 ± 1.4 µmol O_2/min/g dry weight; $P < .001$) compared with sham, and −48.2% ($P < .001$) compared with IR. Similarly, rPoC further impaired V_{max} −50.0% (to 5.1 ± 1.9 µmol O_2/min/g dry weight; $P < .001$) and −40.0% ($P = .001$) compared with sham and IR values, respectively. The differences between PoC and rPoC were not significant.

Concerning skeletal muscle reactive oxygen production, local PoC further increased fluorescence to 13,426 ± 2071, which was significantly higher than sham (207.2%; $P = .002$), and tended to be higher than IR (30.9%; $P = .226$). rPoC further increased the fluorescence to 17,716 ± 6231, which was significantly higher than sham (305.4%; $P < .001$) and significantly higher than IR (72.7%; $P = .011$). The comparison between PoC and rPoC showed no statistical difference.

The evaluation of antioxidant defense showed local PoC increased *SOD1* (110.5%; $P = .020$) and *SOD2* (166.6%; $P = .001$) expressions, but the increase was not significant, being 8.6% ($P = .839$) and 14.2% ($P = .665$) after rPoC compared with sham. *GPx* expression remained decreased after PoC.

Local PoC also increased *Bcl-2* (92.6%; $P = .014$) and normalized the *Bax/Bcl-2* ratio. rPoC increased the expression of the proapoptotic *Bax* gene (391.6%; $P < .001$) and the *Bax/BclII* ratio (621.7%; $P < .001$).

Although interleukin-6 expression was not modified after PoC, immunohistochemical studies revealed presence of inflammation after local PoC and rPoC (Fig 6, *B*).

DISCUSSION

The main results of this study demonstrate for the first time, to our knowledge, (1) that local PoC and ischemic rPoC further increase IR-induced impairments of skeletal muscle mitochondrial oxidative capacity and (2) that these unexpected deleterious effects were associated with enhanced ROS production and inflammation.

IR impaired mitochondrial respiratory chain and increased oxidative stress and genes involved in apoptosis. Besides trauma and lengthy, complicated operations, many pathologic situations, such as arterial embolism, thrombosis, extrinsic compression, or long-lasting circulatory shock, might lead to vascular occlusion or IR episodes in the lower limbs. Because of the relatively high metabolic rate of muscle tissue, a short ischemic interval, followed by reperfusion, might be significantly deleterious.[1-4] Accordingly, we observed decreased muscle oxidative capacity was associated with stimulation of the apoptosis pathway. Indeed, Bax is proapoptotic, enhancing mitochondrial permeability transition pores opening and cytochrome *c* release, and an increased Bax/Bcl-2 ratio has been described as triggering cell apoptosis. Consistently, recent data in mice demonstrated edema, apoptosis, and necrosis after hind limb IR.[28,29]

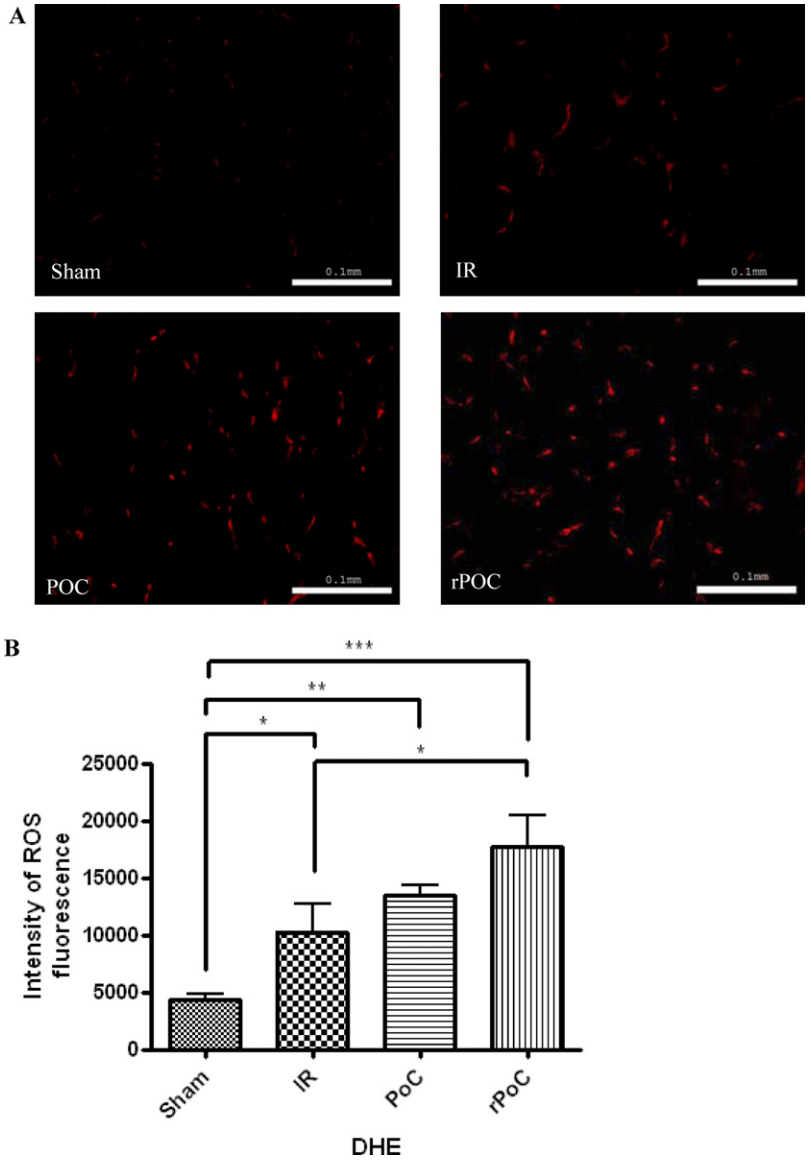

Fig 3. Skeletal muscle production of reactive oxygen species in sham, after ischemia-reperfusion (*IR*), and after local ischemic postconditioning (*PoC*) and remote PoC (*rPoC*). **A,** Fluorescence photographs of each subset. **B,** Intensity of reactive oxygen species (*ROS*) fluorescence. *DHE,* Dihydroethidium. Results are expressed as means ± standard error of the mean (*error bars*). $*P < .05$. $**P < .01$.$***P < .001$.

To further characterize the mechanisms involved, we determined skeletal muscle ROS production and demonstrated a significant oxidative stress increase, likely related to superoxide anion production.[14] Indeed, DHE muscular staining, considered as an efficient marker of ROS production, was increased after IR. Increased production as well as decreased catabolism likely contributed to this oxidative stress enhancement in skeletal muscle. Indeed, impaired mitochondria generate more ROS than normal mitochondria,[14] and reduced antioxidant capacity has been observed in experimental animals and in humans after abdominal aortic aneurysm surgery.[14,22,30] Accordingly, expressions of *SOD1*, *SOD2*, and *GPx*, the main enzymes known to neutralize ROS, were altered after IR. Because of their clinical significance, reducing such muscular alterations remains a challenging problem, particularly in case of unanticipated acute ischemia.

Local ischemic PoC and rPoC further impaired mitochondrial respiratory chain function and increased oxidative stress and inflammation. In this study, unexpectedly, local PoC and rPoC further impaired the muscle, decreasing oxidative capacities and increasing inflammation, compared with sham and IR groups. This first report

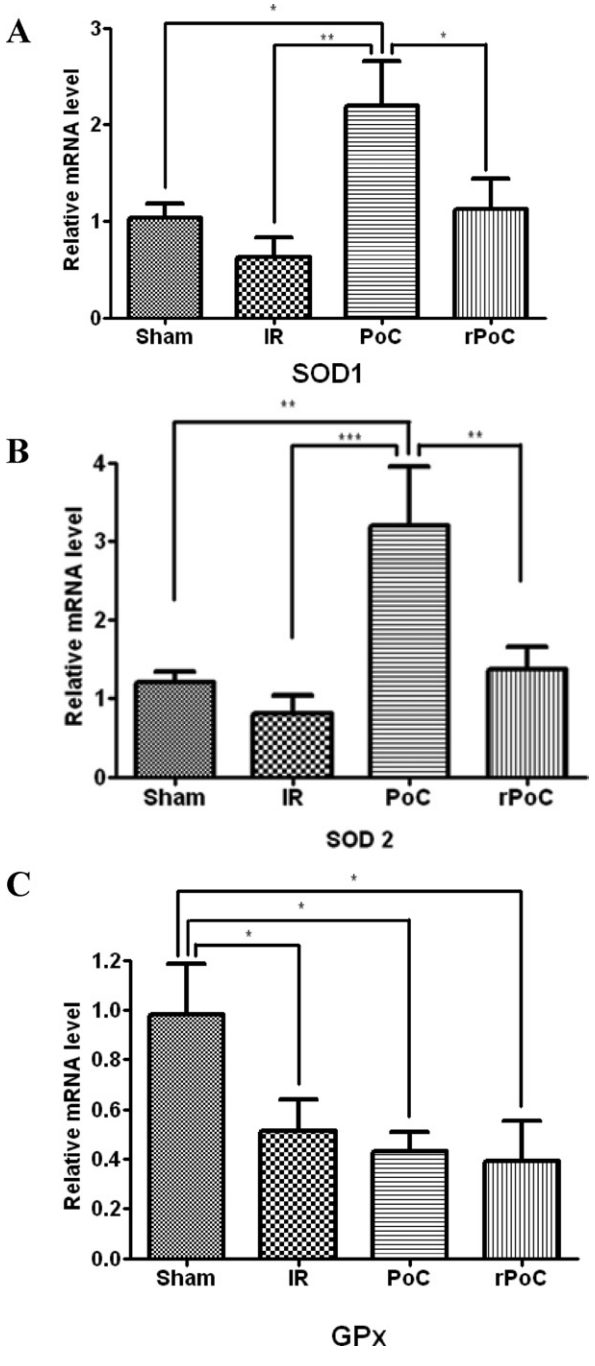

Fig 4. Effects of ischemia-reperfusion (*IR*) and local postconditioning (*PoC*) and remote ischemic postconditioning (*rPoC*) on the expression of genes involved in antioxidant defense. **A,** *Superoxide dismutase 1* (*SOD1*). **B,** *Superoxide dismutase 2* (*SOD2*). **C,** *Glutathione peroxidase* (*GPx*). Results are expressed as means ± standard error of the mean (*error bars*). **P* < .05. ***P* < .01. ****P* < .001.

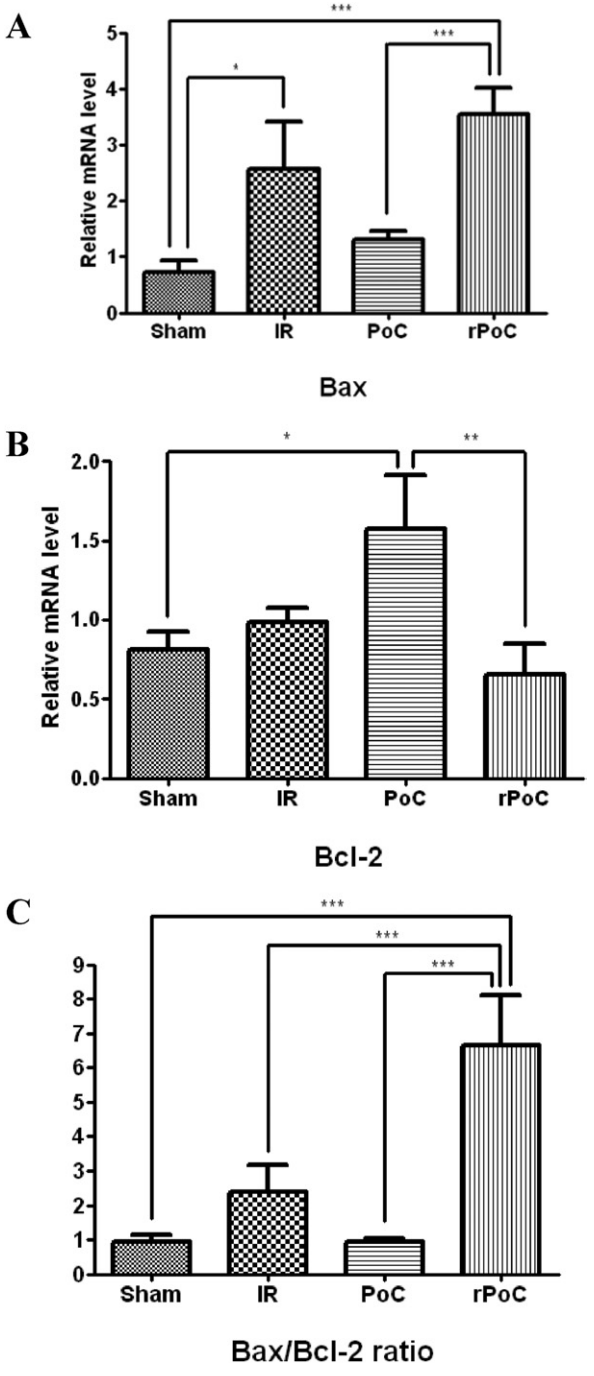

Fig 5. Effects of ischemia-reperfusion (*IR*) and local postconditioning (*PoC*) and remote ischemic PoC (*rPoC*) on the expression of genes involved in apoptosis. **A,** *Bax*. **B,** *BclII*. **C,** Messenger RNA ratio of *Bax/BclII*. Results are expressed as means ± standard error of the mean (*error bars*). **P* < .05. ***P* < .01. ****P* < .001.

of a detrimental effect of PoC on skeletal muscle in the setting of IR appears particularly important. Indeed to date, PoC protocols were thought to be, at worst, inefficient. Interestingly however, PoC deleterious effects have been recently reported in the setting of cardiac IR. Depending on the species and on algorithm used, PoC procedures performed after a short period of ischemia increased myocardial infarct size.[31,32]

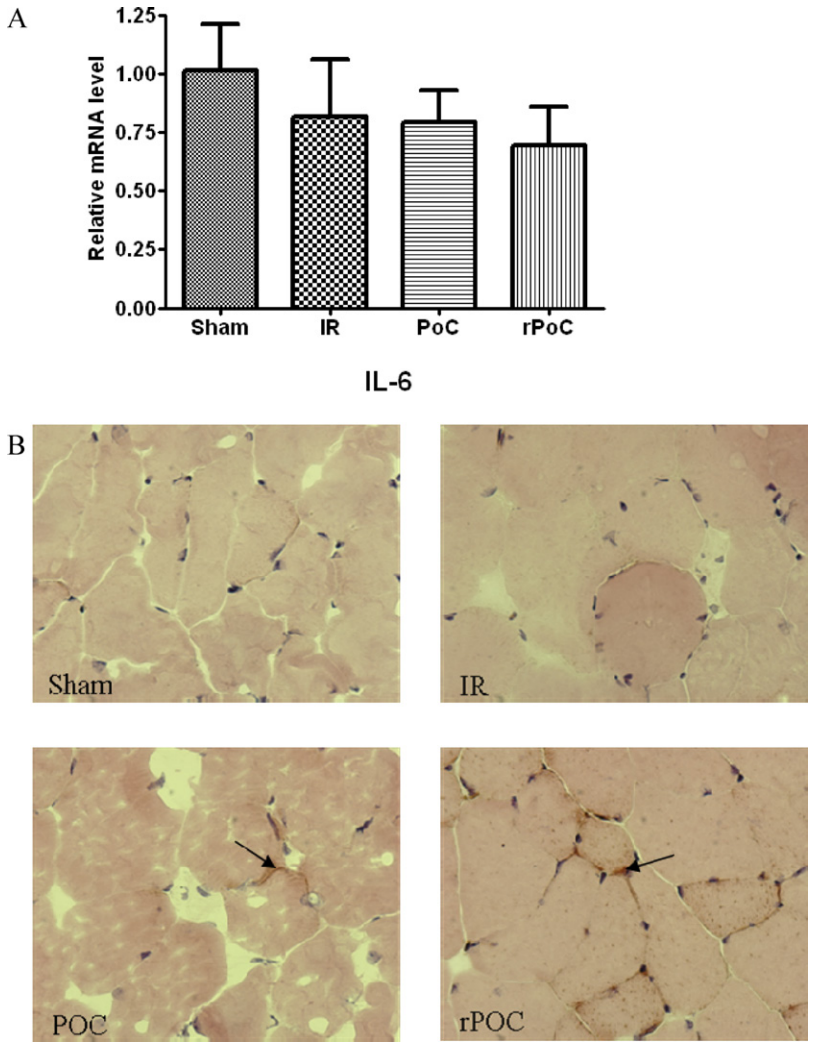

Fig 6. Effects of ischemia-reperfusion (*IR*) and local postconditioning (*PoC*) and remote ischemic PoC (*rPoC*) on inflammation. **A,** Expression of *interleukin-6* (*IL-6*). Results are expressed as means ± standard error of the mean (*error bars*). **B,** Immunohistochemical demonstration of the distribution of macrophages, which are indicated by the *black arrows* (original magnification, ×400).

In our study, however, the index ischemia was similar to that used by Eberlin et al[12] showing mice skeletal muscle protection. PoC might also be unsuccessful if performed too late after IR, when injury is irreversible and mainly related to ischemia alone.[33] Our PoC protocol theoretically fulfilled efficiency criteria because it was performed after 2 hours of ischemia and started at the onset of reperfusion.[10-14] Further, although in our successful previous PoC study[14] we performed three cycles of 1, 2, and 4 minutes of reperfusion, separated by 5 minutes of reocclusion, to allow enough time to the muscle for handling toxic reperfusion products, other data demonstrated that four cycles of 30 seconds of IR also protected the latissimus dorsi in pigs.[10]

An "ideal" PoC protocol remains thus to be established.[31] It might depend on interaction between a number of factors, including duration of ischemia, algorithm of PoC, blood flow stopping sites, phenotype of muscle, sex, age, temperature, and species. Other laboratory-specific unidentified variables might also be in play.[34] Thus, a small change in the conditioning protocol or environment might profoundly modify the outcome. In this view, a protocol in which the total ischemic time is held constant for both groups while applying intermittent bouts of reperfusion in one group should be interesting.

To further understand the mechanisms involved in PoC-related deleterious effects, we think it is noteworthy that local and rPoC were both detrimental. This supports a humoral theory that suggests that remote tissue releases circulating factors acting to modulate the effects of IR on the ischemic organ.[35] Thus, increased oxidative stress and inflammation might have played a key role.

Indeed, DHE muscular staining further increased after local and rPoC. Accordingly, we and others demonstrated the deleterious effects of oxidative stress during skeletal muscle IR and that protection occurs when ROS production is reduced.[14,22] Similarly, we observed muscle inflammation after PoC and rPoC, consistent with previous data demonstrating involvement of inflammation in IR deleterious effects.[29,36] Because leukopenic animals are protected from development of IR,[37] inflammation might be a main cause rather than a consequence of IR injuries.

Although PoC and rPoC both further increased mitochondrial dysfunction, DHE staining and inflammation, protective *Bcl-2*, *SOD1*, and *SOD2* expressions increased after local PoC. Conversely, the proapoptotic pathway was stimulated in the rPoC hind limb. This differential transcriptional response to IR deserves specific investigations. Because ROS production and inflammation increased after local PoC and rPoC in this study, further studies investigating the relationship between oxidative stress and macrophage infiltration might be useful. This might also be analyzed when examining the effect of slowly restoring flow over a given period of time rather than abrupt cessation and reperfusion.

CONCLUSIONS

This study demonstrates that local and remote ischemic postconditioning may both have detrimental effects on skeletal muscle mitochondrial function in the setting of ischemia-reperfusion and that enhanced ROS production was associated with decreased muscle oxidative capacities and inflammation. Thus, although ischemic postconditioning might be a promising therapeutic approach to reduce patient morbidity during major vascular surgeries, caution should be taken before its broader translation to clinical practice.[38] Particularly, the algorithm used should be very well designed and probably personalized to each patient.

We thank Fabienne Goupilleau, Isabelle Bentz, Anne-Marie Leonardo, and Anne Schmitt for their expert biologic and secretarial assistance.

AUTHOR CONTRIBUTIONS

Conception and design: ZM, AC, JB, JZ, BG
Analysis and interpretation: ZM, AC, JB, JP, MK, JM, JZ, BG
Data collection: ZM, AC, JP
Writing the article: ZM, AC, BG
Critical revision of the article: JB, JP, JM, MK, BG
Final approval of the article: ZM, AC, JB, JP, MK, JM, JZ, BG
Statistical analysis: ZM, AC
Obtained funding: BG
Overall responsibility: BG

REFERENCES

1. Miller CC 3rd, Villa MA, Sutton J, Lau D, Keyhani K, Estrera AL, et al. Serum myoglobin and renal morbidity and mortality following thoracic and thoraco-abdominal aortic repair: does rhabdomyolysis play a role? Eur J Vasc Endovasc Surg 2009;37:388-94.
2. Ali ZA, Callaghan CJ, Lim E, Ali AA, Nouraei SA, Akthar AM, et al. Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial. Circulation 2007;116:I98-105.
3. Adembri C, Kastamoniti E, Bertolozzi I, Vanni S, Dorigo W, Coppo M, et al. Pulmonary injury follows systemic inflammatory reaction in infrarenal aortic surgery. Crit Care Med 2004;32:1170-7.
4. Fowkes FG, Anandan CL, Lee AJ, Smith FB, Tzoulaki I, Rumley A, et al. Reduced lung function in patients with abdominal aortic aneurysm is associated with activation of inflammation and hemostasis, not smoking or cardiovascular disease. J Vasc Surg 2006;43:474-80.
5. Martou G, O'Blenes CA, Huang N, McAllister SE, Neligan PC, Ashrafpour H, et al. Development of an in vitro model for study of the efficacy of ischemic preconditioning in human skeletal muscle against ischemia-reperfusion injury. J Appl Physiol 2006;101:1335-42.
6. Thaveau F, Zoll J, Rouyer O, Chafke N, Kretz JG, Piquard F, et al. Ischemic preconditioning specifically restores complexes I and II activities of the mitochondrial respiratory chain in ischemic skeletal muscle. J Vasc Surg 2007;46:541-7.
7. Crisostomo PR, Wairiuko GM, Wang M, Tsai BM, Morrell ED, Meldrum DR. Preconditioning versus postconditioning: mechanisms and therapeutic potentials. J Am Coll Surg 2006;202:797-812.
8. Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, et al. Transient limb ischemia induces remote ischemic preconditioning in vivo. Circulation 2002;106:2881-3.
9. Beyersdorf F, Schlensak C. Controlled reperfusion after acute and persistent limb ischemia. Semin Vasc Surg 2009;22:52-7.
10. McAllister SE, Ashrafpour H, Cahoon N, Huang N, Moses MA, Neligan PC, et al. Postconditioning for salvage of ischemic skeletal muscle from reperfusion injury: efficacy and mechanism. Am J Physiol Regul Integr Comp Physiol 2008;295:R681-9.
11. Gyurkovics E, Aranyi P, Stangl R, Onody P, Ferreira G, Lotz G, et al. Postconditioning of the lower limb–protection against the reperfusion syndrome. J Surg Res 2011;169:139-47.
12. Eberlin KR, McCormack MC, Nguyen JT, Tatlidede HS, Randolph MA, Austen WG Jr. Sequential limb ischemia demonstrates remote postconditioning protection of murine skeletal muscle. Plast Reconstr Surg 2009;123:8S-16S.
13. Park JW, Kang JW, Jeon WJ, Na HS. Postconditioning protects skeletal muscle from ischemia-reperfusion injury. Microsurgery 2010;30:223-9.
14. Charles AL, Guilbert AS, Bouitbir J, Goette-Di Marco P, Enache I, Zoll J, et al. Effect of postconditioning on mitochondrial dysfunction in experimental aortic cross-clamping. Br J Surg 2011;98:511-6.
15. Tapuria N, Kumar Y, Habib MM, Abu Amara M, Seifalian AM, Davidson BR. Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury—a review. J Surg Res 2008;150:304-30.
16. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003;285:H579-88.
17. Mansour Z, Bouitbir J, Charles AL, Talha S, Kindo M, Pottecher J, et al. Remote and local ischemic preconditioning equivalently protects rats skeletal muscle mitochondrial function during experimental aortic cross-clamping. J Vasc Surg 2012;55:497-505.e1.
18. Chaudry IH, Bland KI. Cellular mechanisms of injury after major trauma. Br J Surg 2009;96:1097-8.
19. Pipinos II, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, et al. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. Free Radic Biol Med 2006;41:262-9.
20. Zoll J, Sanchez H, N'Guessan B, Ribera F, Lampert E, Bigard X, et al. Physical activity changes the regulation of mitochondrial respiration in human skeletal muscle. J Physiol 2002;543:191-200.
21. Pipinos II, Boska MD, Shepard AD, Anagnostopoulos PV, Katsamouris A. Pentoxifylline reverses oxidative mitochondrial defect in claudicating skeletal muscle. J Surg Res 2002;102:126-32.

22. Tran TP, Tu H, Pipinos II, Muelleman RL, Albadawi H, Li YL. Tourniquet-induced acute ischemia-reperfusion injury in mouse skeletal muscles: involvement of superoxide. Eur J Pharmacol 2011;650: 328-34.
23. Thaveau F, Zoll J, Bouitbir J, N'Guessan B, Plobner P, Chakfe N, et al. Effect of chronic pre-treatment with angiotensin converting enzyme inhibition on skeletal muscle mitochondrial recovery after ischemia/ reperfusion. Fundam Clin Pharmacol 2010;24:333-40.
24. Gupta K, Aggarwal N, Rao M, Verma UC, Anand R. Re-emphasizing the importance of tourniquet time: severe myocardial depression following tourniquet deflation. Acta Anaesthesiol Scand 2008;52:873.
25. Noll E, Bouitbir J, Collange O, Zoll J, Charles AL, Thaveau F, et al. Local but not systemic capillary lactate is a reperfusion biomarker in experimental acute limb ischaemia. Eur J Vasc Endovasc Surg 2012;43:339-40.
26. Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, et al. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. Mol Cell Biochem 1998;184:81-100.
27. Zoll J, Monassier L, Garnier A, N'Guessan B, Mettauer B, Veksler V, et al. ACE inhibition prevents myocardial infarction-induced skeletal muscle mitochondrial dysfunction. J Appl Physiol 2006;101:385-91.
28. Henderson PW, Jimenez N, Ruffino J, Sohn AM, Weinstein AL, Krijgh DD, et al. Therapeutic delivery of hydrogen sulfide for salvage of ischemic skeletal muscle after the onset of critical ischemia. J Vasc Surg 2011;53:785-91.
29. Tsubota H, Marui A, Esaki J, Bir SC, Ikeda T, Sakata R. Remote postconditioning may attenuate ischaemia-reperfusion injury in the murine hindlimb through adenosine receptor activation. Eur J Vasc Endovasc Surg 2010;40:804-9.
30. Westman B, Johansson G, Luo JL, Söderlund K, Wernerman J, Hammarqvist F. Effects on skeletal muscle glutathione status of ischemia and reperfusion following abdominal aortic aneurysm surgery. Ann Vasc Surg 2006;20:99-105.
31. Skyschally A, van Caster P, Iliodromitis EK, Schulz R, Kremastinos DT, Heusch G. Ischemic postconditioning: experimental models and protocol algorithms. Basic Res Cardiol 2009;104:469-83.
32. Manintveld OC, Te Lintel Hekkert M, van den Bos EJ, Suurenbroek GM, Dekkers DH, Verdouw PD, et al. Cardiac effects of postconditioning depend critically on the duration of index ischemia. Am J Physiol Heart Circ Physiol 2007;292:H1551-60.
33. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ, et al. Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 2010;87:406-23.
34. Iliodromitis EK, Downey JM, Heusch G, Kremastinos DT. What is the optimal postconditioning algorithm? J Cardiovasc Pharmacol Ther 2009;14:269-73.
35. Walsh SR, Tang TY, Sadat U, Gaunt ME. Remote ischemic preconditioning in major vascular surgery. J Vasc Surg 2009;49:240-3.
36. Formigli L, Lombardo LD, Adembri C, Brunelleschi S, Ferrari E, Novelli GP. Neutrophils as mediators of human skeletal muscle ischemia-reperfusion syndrome. Hum Pathol 1992;23:627-34.
37. Sheu EG, Oakes SM, Ahmadi-Yazdi C, Afnan J, Carroll MC, Moore FD Jr. Restoration of skeletal muscle ischemia-reperfusion injury in humanized immunodeficient mice. Surgery 2009;146:340-6.
38. Freixa X, Bellera N, Ortiz-Pérez JT, Jiménez M, Paré C, Bosch X, et al. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. Eur Heart J 2012;33:103-12.

Submitted Nov 3, 2011; accepted Jan 31, 2012.

Additional material for this article may be found online at www.jvascsurg.org.

Table I. (online only). Primer sequences used for quantitative real-time polymerase chain reaction (RT-PCR) amplification

Target gene	*Accession number*	*Forward primer 5′ → 3′*	*Reverse primer 5′ → 3′*
SOD1	NM017050	AGATGACTTGGGCAAAGGTG	CAATCCCAATCACACCACAA
SOD2	NM017051	CTGGACAAACCTGAGCCCTA	GAACCTTGGACTCCCACAGA
GPx	NM030826	GCCGAGTGTGGTTTACGAAT	GGCTGCAAACTCCTTGATTT
Bax	NC005100	GCTGGACACTGGACTTCCTC	GAGGACTCCAGCCACAAAGA
Bcl-2	NC005112	CGACTTTGCAGAGATGTCCA	CCTGAAGAGTTCCTCCACCA
IL-6	NM012589	TACCCCAACTTCCAATGCTC	GGTTTGCCGAGTAGACCTCA
β-actin	BC063166	GCACAACTTCAGCCTCCCAGA	CTTCCCATTCACCGCTCCATT

GPx, Glutathione peroxidase; *IL-6,* interleukin-6; *SOD,* superoxide dismutase.

Table II. (online only). Relative messenger RNA expression levels of superoxide dismutase (*SOD*) 1 and 2, glutathione peroxidase (*GPx)*, Bax, Bcl-2, Bax/Bcl-2 ratio, and interleukin-6 (*IL-6*)

Gene	*Sham*	*IR group*	*PoC group*	*rPoC group*
SOD1	1.04 ± 0.32	0.63 ± 0.45	2.19 ± 1.04[a,b]	1.13 ± 0.78[c]
SOD2	1.20 ± 0.33	0.82 ± 0.45	3.20 ± 1.31[d,e]	1.37 ± 0.67[f]
GPx	0.98 ± 0.49	0.51 ± 0.28[a]	0.43 ± 0.19[a]	0.39 ± 0.40[a]
Bax	0.72 ± 0.50	2.56 ± 1.70[d]	1.31 ± 0.36	3.54 ± 1.16[f,g]
Bcl-2	0.82 ± 0.28	0.98 ± 0.21	1.58 ± 0.75[a]	0.66 ± 0.43[f]
Bax/Bcl-2	0.92 ± 0.49	2.40 ± 1.50	0.92 ± 0.27	6.64 ± 2.95[g,h,i]
Il-6	1.01 ± 0.48	0.82 ± 0.54	0.79 ± 0.33	0.70 ± 0.40

IR, Ischemia-reperfusion; *PoC,* local postconditioning; *rPoC,* remote postconditioning.
[a] $P < .05$ compared with sham.
[b] $P < .05$ compared with IR.
[c] $P < .05$ compared with PoC.
[d] $P < .01$ compared with sham.
[e] $P < .001$ compared with IR.
[f] $P < .01$ compared with PoC.
[g] $P < .001$ compared with sham.
[h] $P < .01$ compared with IR.
[i] $P < .001$ compared with PoC.